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# AN ULTRASTRUCTURAL STUDY OF THE DEVELOPMENT OF RADIATION INJURY IN THE LUNG

by

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## ADMINISTRATIVE INFORMATION

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## ABSTRACT

1. Radiation doses of 2000 R were given to the left hemithorax of a group of 25 rats. At intervals of from one hour to one year following irradiation sections of the lung were examined with the electron microscope. The initial site of radiation damage appears to lie chiefly in the endothelium. The endothelium is sloughed and the original endothelial space is replaced by collagen and mast cell infiltrates. Some capillaries are recanalized by new endothelial cells. Eventually these capillaries attain an appearance similar to that of the original capillary but with a slightly thickened endothelium and basement membrane. If the original capillary architecture is not maintained, massive fibrosis results.

2. The mast cell participates extensively in the repair of the radiation damage and is closely associated with collagen and new capillary formation.

3. It is stressed that the degree of damage occurring after a given dose of irradiation varies widely and that these observations were made on only small samples of lungs.

## SUMMARY

The Problem:

The lung contains one of the richest capillary networks in the body. Its response to radiation should shed light on the response of capillaries in other parts of the body. A better understanding of the nature of radiation injury and the repair of this injury in the lung could lead to a better understanding of late radiation changes following total body exposures. These studies were conducted at intervals of one day to one year following delivery of 2000 R to the left hemithorax of rats. Samples were studied by means of the electron microscope.

The Findings:

The results indicate that the initial site of radiation injury in the lung at moderate dose levels is in the endothelial cells. Extensive endothelial loss occurs at two to three months following irradiation and the endothelial spaces are filled with collagen and mast cells. Some of the spaces are then recanalized and the resultant capillary has a slightly thickened endothelium and basement membrane. A wide variation was noted in the degree of damage within a lung and between lungs from different animals. Some areas remained unchanged even at one year following irradiation, whereas, in other lungs extensive breakdown and fibrosis was noted.

# INTRODUCTION

The lung represents a dose-limiting structure in the radiation treatment of thoracic and chest wall lesions. It contains one of the richest capillary networks in the body. Thus, radiation-induced changes noted within the lung indicate reaction of small vessels in other tissues. Information concerning capillary reactions would lead to a better understanding of residual injury after total body irradiations.

Changes seen in the lung after therapeutic and experimental doses of radiation have been described in several extensive reports (3,4,9). Of the experimental studies on radiation pneumonitis, that of Jennings and Arden (3) is one of the more recent. These studies were limited by the resolution of the light microscope, which did not allow visualization of the detailed structure of the alveolar capillary wall. Neither did it permit accurate identification of the cell types involved in reaction to injury.

Detailed examination of the various components of the alveolar capillary wall was believed possible with the use of the electron microscope. With this instrument, the specific cells involved in the reaction to radiation and in repair of injury could be studied. It

would also enable detailed study of the structure of the repaired lung.

## MATERIALS AND METHODS

Twenty-five male Sprague-Dawley rats from the specific pathogen-free colony of the U. S. Naval Radiological Defense Laboratory were used. The rats were four months of age and free of any pulmonary infections. Irradiation was performed through a left anterior chest field of 5 X 2.5 cm., using 250 kvp radiation with a HVL of 1.28 mm Cu. The animals were anesthetized with Nembutal for the irradiation and their bodies shielded with lead of one-fourth inch thickness. The irradiated volume included the left lung and mediastinum, but not the right lung. A dose of 2000 R was delivered to the midplane of the left chest.

Animals were killed at various periods, ranging from one hour to 12 months after irradiation. Control animals were taken at random from each group and their lungs prepared in a manner similar to that used in the irradiated animals.

For removal of the specimens, ether anesthesia was used and the left hemithorax was opened rapidly. While the animal was still breathing, a small portion of the peripheral zone of the left upper lobe was removed. This specimen was quickly minced in a solution of one per cent osmium tetroxide, buffered with veronal acetate and

containing sucrose, according to the method of Caulfield (1). The tissues were fixed for one hour at room temperature and then embedded in Araldite, as described by Luft (7). Sections were cut with a Porter-Bloom MT-1 microtome using glass knives, mounted unsupported on 200-mesh grids and stained with lead (8) and uranyl acetate. The sections were examined and photographed in an RCA EMU 3 G electron microscope.

## RESULTS

In the control animals, the capillary structure, as previously described (6), was seen with thin walls and occasional septal and "dust" cells (Figure 1). The epithelium was separated from the endothelium by a basement membrane about 0.16 micron thick.

In the animals given 2000 R and sacrificed at close intervals, no alteration was noted immediately after the irradiation, the first changes being seen one day later. The alterations noted at first were not widespread and involved only a few per cent of the observed capillaries. The change consisted chiefly of segmental separation of the endothelium from the basement membrane and scattered vacuoles or blebs within the endothelium. No damage was noted in the epithelial covering or in the basement membrane. The changes were less marked at one month and six weeks after the irradiation. Beginning at two months from the time the study was initiated, the endothelial changes became widespread with frequent vacuole formation and endothelial

sloughing (Figure 2). Associated with this was apparent obstruction of capillary spaces by separated endothelium. The basic structure of the alveolus was maintained with no alteration noted in the epithelium or in the basement membrane during this phase. These changes became more marked at three months and were associated with the appearance of plasma cells within the alveolar speta. During the period from three to six months after irradiation, a dynamic process appeared to be occurring, which varied in its progress from one alveolus to another. This process consisted of the complete loss of the endothelial lining of the alveolar capillaries. Following this period, plasma cells appeared in the interstitial space. Numerous mast cells then appeared, intimately associated with collagen. These completely obliterated the previous capillary space while the original basement membrane and epithelial covering were maintained. During this process, the general architecture of the lung remained relatively unchanged, with the relationship of the previous capillary spaces and the alveoli remaining constant (Figure 3).

In areas in which mast cells infiltrated and collagen proliferation was seen, there also appeared to be buds of new capillaries growing into the previous capillary space (Figure 4). They consisted first of cells resembling endothelial cells without a lumen. These cells became rounded and a lumen appeared in the center. The cell was

surrounded by a separate basement membrane, distinct from that remaining under the epithelial lining of the alveolus. These vessels expanded, nearly filling the old alveolar capillary lumen, but a somewhat thickened basement membrane remained (Figure 5). The cytoplasm of these cells was more abundant than the original. These new capillaries resembled connective tissue vessels which had grown into the old endothelial space and again carried blood. In some of the original capillaries, complete replacement with collagen was seen and no new vessels appeared to grow in. Duplicate specimens were taken in a period from three to six months in additional animals. In some of these, even at 2000 R, areas of breakdown were seen in the thin alveolar capillary structure. With this breakdown, there were dissolution of the normal basement membrane and cellular infiltrate with additional mast and epithelial cells. Often, a large amount of fluid and markedly excessive collagen resulted in a greatly widered alveolar septum. In a few animals, large infiltrates and collections of alveolar macrophages were seen. In general, however, at the 2000 R level, the changes were limited to the endothelial lining and its replacement. Specimens taken at six months to one year after irradiation showed diminution in the number of mast cells. Finally, a moderate thickening of the endothelial cells secondary to replacement by connective tissue-type vessels and collagen replacement of some of the old

capillary lumena resulted. Increase in thickness of basement membrane was seen at 10 and 12 months after irradiation (Figure 6).

## DISCUSSION

Extensive reviews have been published which described the changes in human lungs after radiation therapy. Those of Jennings and Arden (4) and of Warren (9) described initial alterations consisting of non-specific edema, congestion, and atelectasis. The authors stated that the lesions believed to be secondary to radiation consisted of fibrin exudate in the alveoli, epithelial proliferation, and fibrillar thickening of the alveolar septa. In addition, an increased cellularity of the alveolar septa and fibrosis had been noted. Proliferation within the larger blood vessels, particularly endarteritis, had been noted also. Most of the peripheral changes in the lung that have been described were epithelial changes. Most human material is obtained relatively late after irradiation and is associated with secondary factors, such as infection, cardiac disease, and advanced malignancy.

An extensive investigation reported by Jennings and Arden (3), described the changes in rat lungs after irradiation to the entire thorax (3000 R). Many of the animals died. Possibly the changes were more marked and occurred earlier because both lungs had been irradiated simultaneously. These authors described marked congestion in the alveolar septa four days after irradiation, with pulmonary edema

developing by the twelfth day. Early fibrillar thickening of the septa occurred at 17 days. By the sixtieth day, massive septal thickening was present, secondary to edema and to reticulum and cellular infiltrates. One year after irradiation, marked septal thickening was seen. Epithelial overgrowth and migration with displacement of the capillaries to the surface of the thickened septa were also noted at that time.

The electron microscope allows a much more detailed investigation of the specific lesions that develop after irradiation. Its disadvantage is that only small areas of sampling within the lung and a small number of samples can be studied. Since the initiation of this investigation, two reports (2,5) of the use of the electron microscope have described radiation changes in the lungs of animals after a single-dose irradiation to the thorax. One of these (5) reported that the first sign of damage appeared in the endothelium after doses of 4000 R. The study was terminated at 100 days, however, before wisespread late changes had occurred. An experiment was performed in rabbits by Garbagni, Chiarle, and Bellion (2), who described the effect of 4000 R, using electrons from a 31 Mev betatron with specimens taken at intervals up to 40 days after irradiation. They emphasized the proliferation of connective tissue, which extended even into the alveolar walls, but did not describe endothelial degeneration.

The present study describes changes following irradiation of the

lung in greater detail and for a much longer period than previously reported. It must be emphasized, however, that damage occurred after 2000 R, even in this small group, varied widely, and that only very small portions of the lung were examined in the electron microscope. A reasonably clear picture of the pathogenesis of radiation damage and repair was obtained. However, the damage is expressed initially by the endothelial cells, which begin to reveal signs of cell death as early as one day after irradiation. The number of cells with these changes was small. Extensive changes were not noted until two months after irradiation when an endothelial loss began in the lung. Damage was expressed first by the formation of blebs and by separation of sections of endothelium from the basement membrane. Not all of the capillaries are involved at one time. Apparently, the blood is able to course through those unaffected, while other areas are being damaged. After the loss of the endothelium, plasma cells appear, then mast cells, and the ingrowth of new collagen. When basement membrane and epithelial covering are intact and infection is absent, new blood vessels apparently then grow into the old alveolar capillary space. These changes result in recanalization. Some of the capillaries are not recanalized and remain as fibrotic strands. Apparently, from the specimens obtained at ten months and at one year, the resultant reformed alveolar capillaries are somewhat dilated and their endothelia somewhat thickened.

The basement membrane is thickened as much as four times normal in some areas and contains collagen fibers. Initially, the new capillaries give the appearance of definite connective tissue vessels with their own basement membrane. At later dates they have been transformed into capillaries similar to the type found in the lung.

The changes described do not occur in all areas of the lung and are not present in all specimens. They may be much more severe, perhaps because of intervening atelectasis or infection. The role of the mast cell in the repair process appears extensive but whether it produces collagen or is only associated with healing is not clear. When more extensive damage is present, the barrier of the epithelial basement membrane is broken down. This allows an enlargement of the spetum with extensive mast cell and septal cell proliferation and collagen production. Consolidation can be massive, resembling a sheet of connective tissue with complete loss of the original architecture. When extensive damage has occurred, large numbers of alveolar macrophages are seen in the area. With intact basic alveolar architecture, no macrophage infiltrates are noted. In these instances, the cells seen chiefly are plasma cells associated with the initial damage, and mast cells associated with the repair and recanalization phases.

## SUMMARY

Radiation doses of 2000 R were given to the left hemithorax of

rats. At intervals from one hour to one year after irradiation, the lungs were studied with the electron microscope.

The initial site of radiation damage appears to lie chiefly in the endothelium. The endothelium is sloughed and the original endothelial space replaced by collagen and mast cell infiltrate. Some capillaries are recanalized by new endothelial cells. Eventually they attain an appearance similar to that of the original capillary but with a somewhat thickened endothelium and basement membrane. If the original capillary alveolar architecture is not maintained, massive fibrosis results with an obviously nonfunctional lung. When it is maintained and recanalization occurs, a functional unit results.

The mast cell participates greatly in the repair of the radiation damage and is closely associated with collagen and new capillary formation.

It is stressed that the degree of damage occurring after a given dose varies widely and that these observations were made on only small samples of lungs.

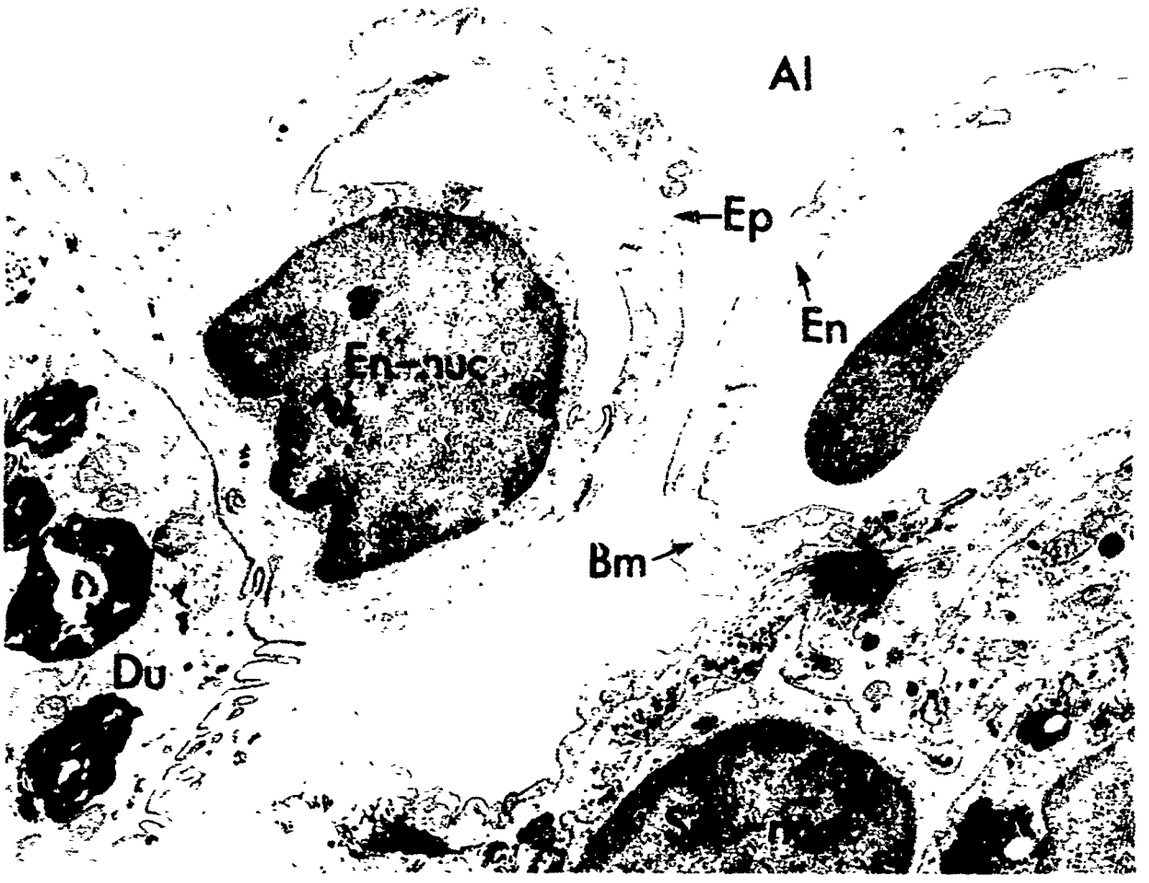


Fig. 1. Normal adult rat lung. Note the nucleus of an endothelial cell (En-nuc) and of a septal cell (Sep-nuc). A "dust cell" (Du) is adjacent to 1 of the 2 alveolar capillaries. The basement membrane (Bm) of the capillaries is covered by an inner layer of endothelium (En) and an outer layer of epithelium (Ep) which are thin in area adjacent to the alveolus (Al). 7,000 X.

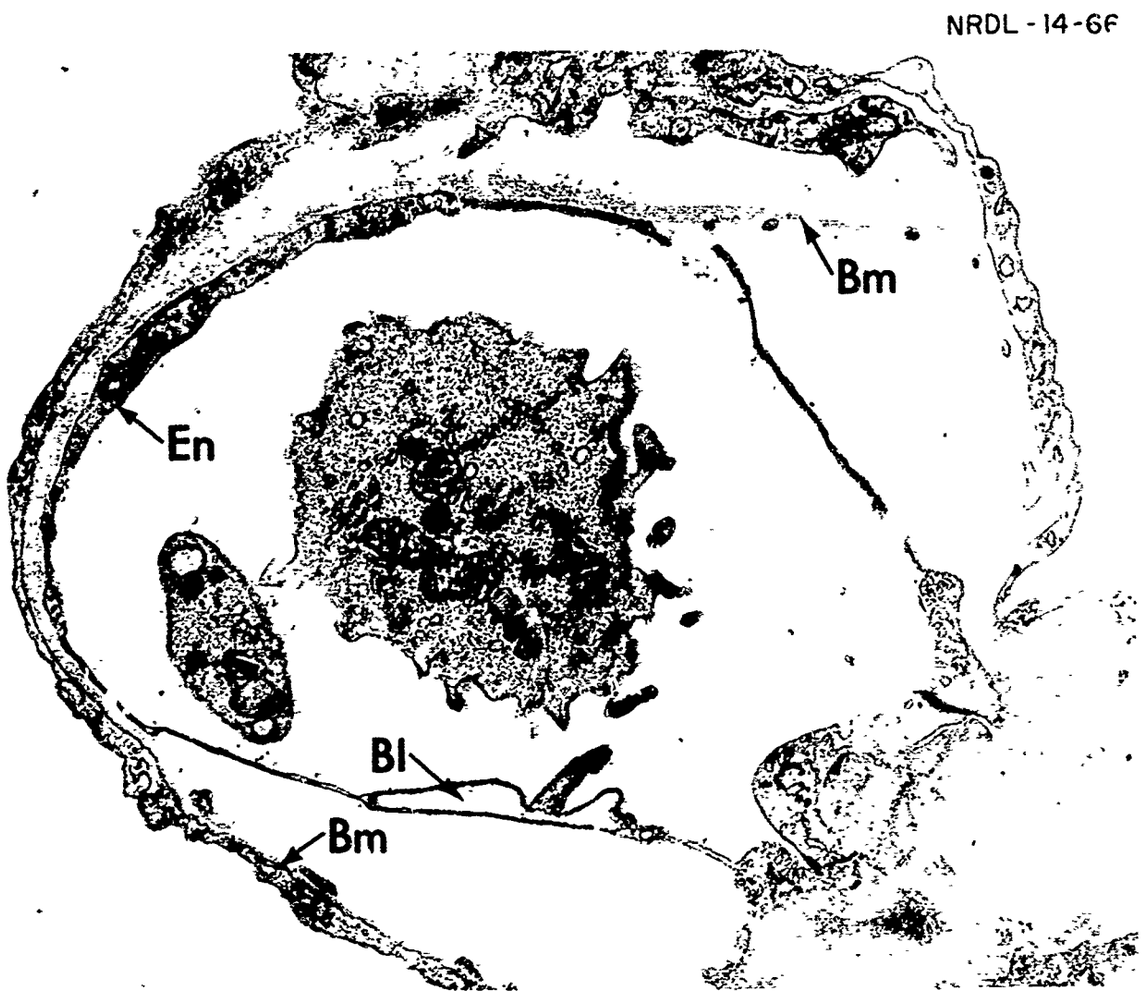


Fig. 2. Rat lung 2 months after 2,000 R. Note that the endothelium (En) has formed a bleb (Bl) and is separated from the basement membrane (Bm) in most of its circumference. 10,000X.

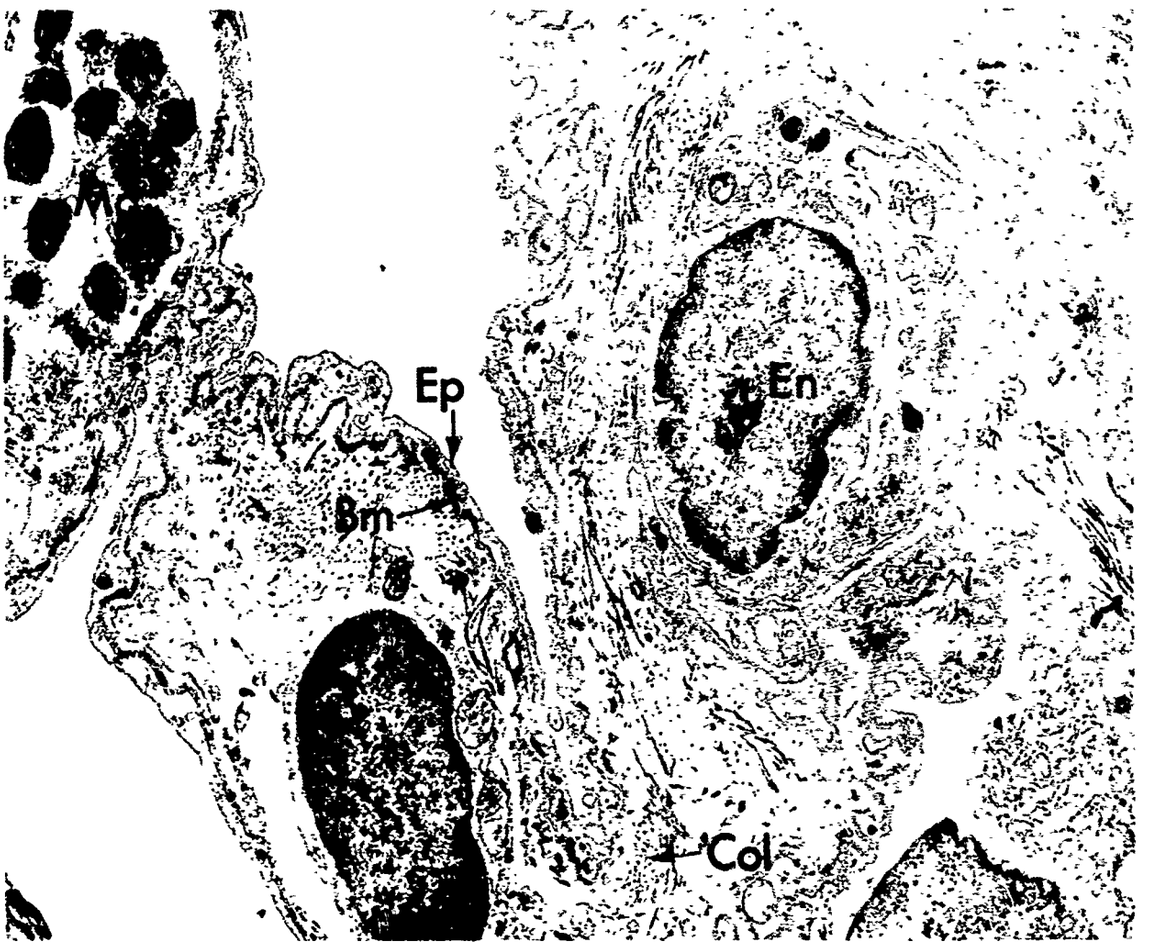


Fig. 3. Rat lung 6 months after 2,000 R. Note that the alveolar capillary has an intact epithelium (Ep) and basement membrane (Bm). The vascular lumen has been replaced by a mast cell (Ma), collagen (Col), and endothelial cells (En), one of which shows early canalization. 7,000 X.

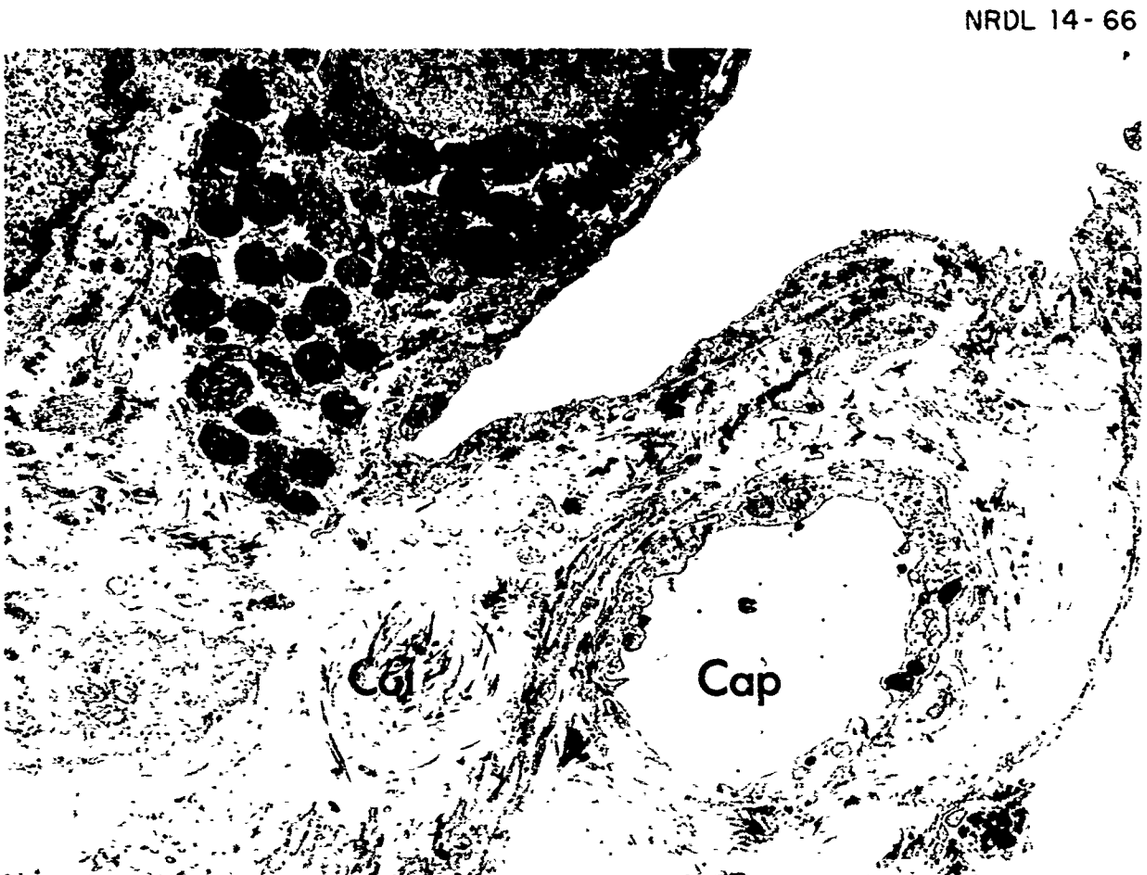


Fig. 4. Rat lung 6 months after 2,000 R. Note the intace epithelium (Ep) and basement membrane (Bm). The original endothelium (En) is absent. The lumen contains collagen (Col), a mast cell (Ma), and a new capillary (Cap). 6,000 X.

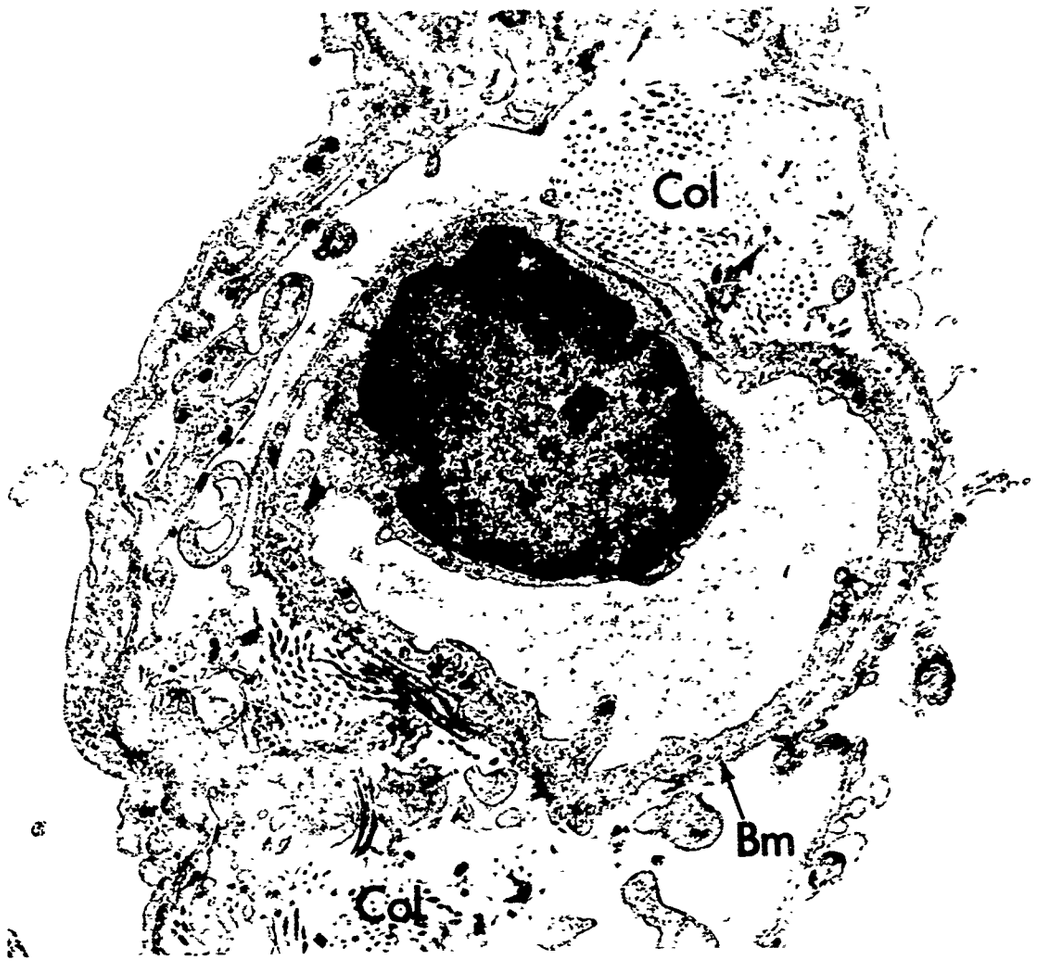


Fig. 5. Rat lung 8 months after 2,000 R. A new capillary (Cap) has enlarged to fill the original lumen. A second basement membrane (Bm) and excess collagen (Col) remain. 7,000 X.

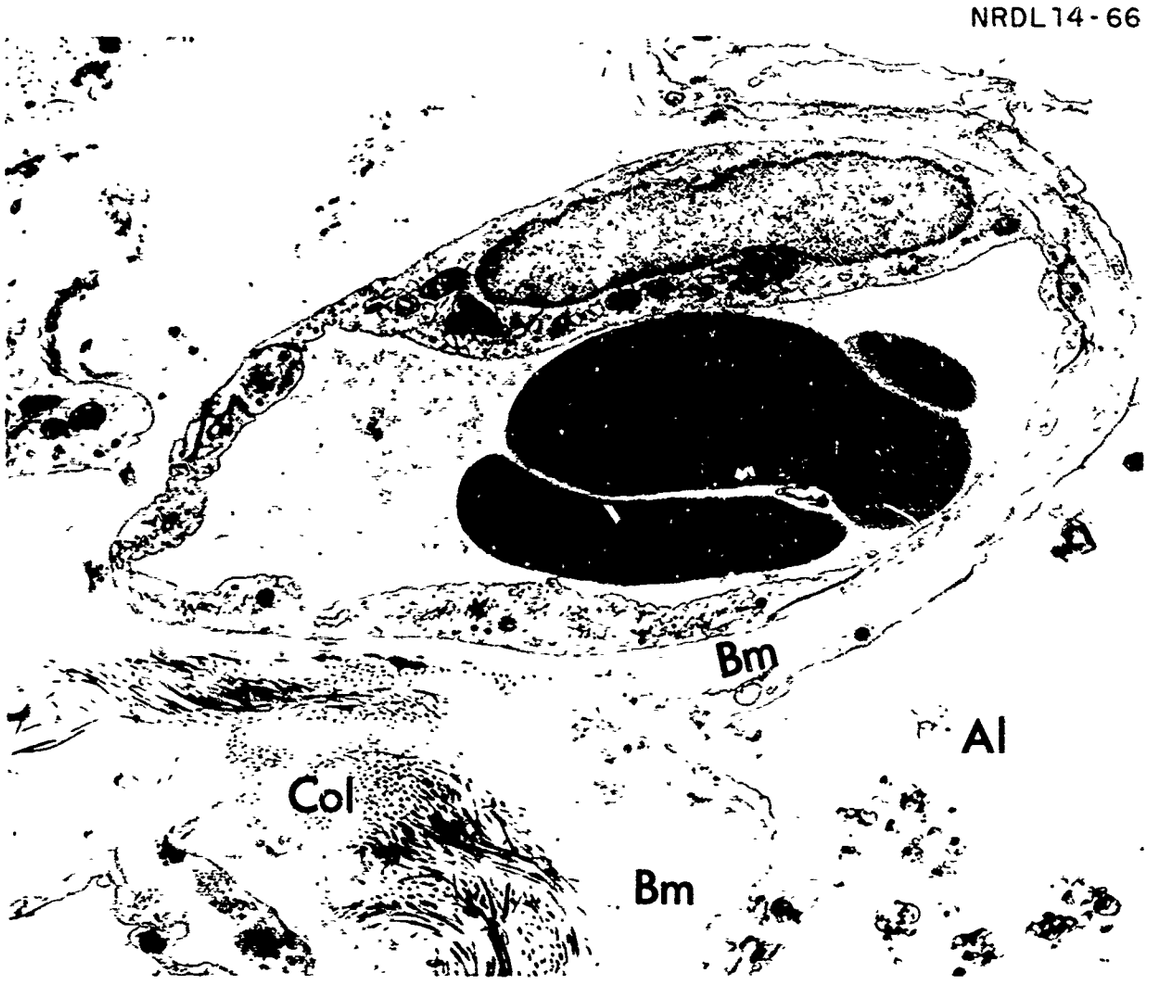


Fig. 6. Rat lung 10 months after 2,000 R. The epithelium (Ep) and basic architecture of capillary (Cap) remains. A portion of the lumen is filled with collagen (Col) and excess basement membrane material (Bm). Note the marked residual thickening of the basement membrane (Bm) around the capillary (Cap). The alveolar space (Al) contains some debris. 7,000 X.

## REFERENCES


1. J. B. Caulfield: Effects of Varying the Vehicle for $O_sO4$ in Tissue Fixation. J. Biophys. Biochem. Cytol. 3:827-830, 1957.
2. R. Garbagni, S. Chiarle, and B. Bellion: Osservazioni sull' ultrastruttura del polmone irradiato. Minerva Nucl. 8:245-252, 1964.
3. F. L. Jennings and A. Arden: Development of Experimental Radiation Pneumonitis. Arch. Path. 71:437-446, 1961.
4. F. L. Jennings and A. Arden: Development of Radiation Pneumonitis. Time and Dose Factors. Arch. Path. 74:351-360, 1962.
5. F. L. Jennings and R. A. Turner: Radiosensitivity of Epithelium and Endothelium in the Lungs. Rad. Res. 22:201, 1964.
6. H. E. Karrer: Ultrastructure of Mouse Lung; General Architecture of Capillary and Alveolar Walls. J. Biophys. Biochem. Cytol. 2: 241-252, 1956.
7. J. H. Luft: Improvements in Epoxy Resin Embedding Methods. J. Biophys. Biochem. Cytol. 9:409-414, 1961.
8. E. S. Reynolds: The Use of Lead Citrate at High pH as an Electron-opaque Stain in Electron Microscopy. J. Cell. Biol. 17:208-212, 1963.
9. S. Warren: Effects of Radiation on Normal Tissues. Arch. Path. 34:917, 1942.

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